Insulin in Combination with N-Acetylcysteine Protects Hypoxia-Induced Toxicity in 661W Cells

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Background: Proliferative diabetic retinopathy (PDR) is the leading cause of blindness among working-age adults. Photoreceptors are the most numerous and metabolically demanding cells in the retina thus oxygen is essential for retinal function. It has been reported that photoreceptors found in rat retina are specifically vulnerable to hypoxia. Hypoxia-induced metabolic stress leads to photoreceptor atrophy and retinopathy. Furthermore, photoreceptor cell death is known to occur mainly through apoptosis. However, the protection of hypoxia-induced-cytotoxicity in cone photoreceptor cells has not been investigated extensively. The aim of this study was to determine whether co-treatment of insulin and the N-Acetyl-L-Cysteine (NAC) (a free radical scavenger) efficiently protects against hypoxia-induced cytotoxicity in 661W cells.

Methods: 661W, an immortalized mouse cone photoreceptor cells, were cultured at 5% CO_2 at 37˚C in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS, penicillin (100 units/mL), and streptomycin (100µg/mL). Cobalt (II) Chloride hexahydrate ($CoCl_2$) was used to induce hypoxia. Insulin was suspended in sterile water, and NAC was diluted in the culture medium. For recovery experiment, cells were pretreated with $CoCl_2$ for 24hrs, and then followed by replacing of medium with insulin (100nM) and NAC (3mM) alone, or with a combination of the two reagents for another 24hrs. Cell viability was determined by MTT assay in a 96 well culture plate. Morphological changes of the cells were observed and photographed under phase-contrast microscope and protein expression was measured by Western blot analysis. Statistical analysis was undertaken using independent two-tailed Students' t-test and determined with SPSS Statistics software.

Results: Treatment with $CoCl_2$ significantly inhibited cell proliferation, reduced the number of viability cells, and induced apoptosis, initiated (poly (ADP-ribose) polymerase (PARP) cleavage, and increased caspase 3 activation. In addition, $CoCl_2$ treatment led to oxidative stress, autophagy, and ubiquitination in the 661W cells. All of these effects, including cell proliferations were significantly reversed by the combination treatment of Insulin and NAC. In contrast, treatment with Insulin alone did not result in a similar protective effect and NAC partially protects against hypoxia induced toxicity.

Conclusion: Hypoxia induces significant apoptosis, oxidative stress, and protein ubiquitination in 661W cone photoreceptors. A combination treatment of Insulin and NAC completely reversed such hypoxia-induced cytotoxicity. Additional research on a combination therapy employing insulin and NAC may provide a novel and promising therapeutic strategy for hypoxia-mediated cone photoreceptor cell damage.